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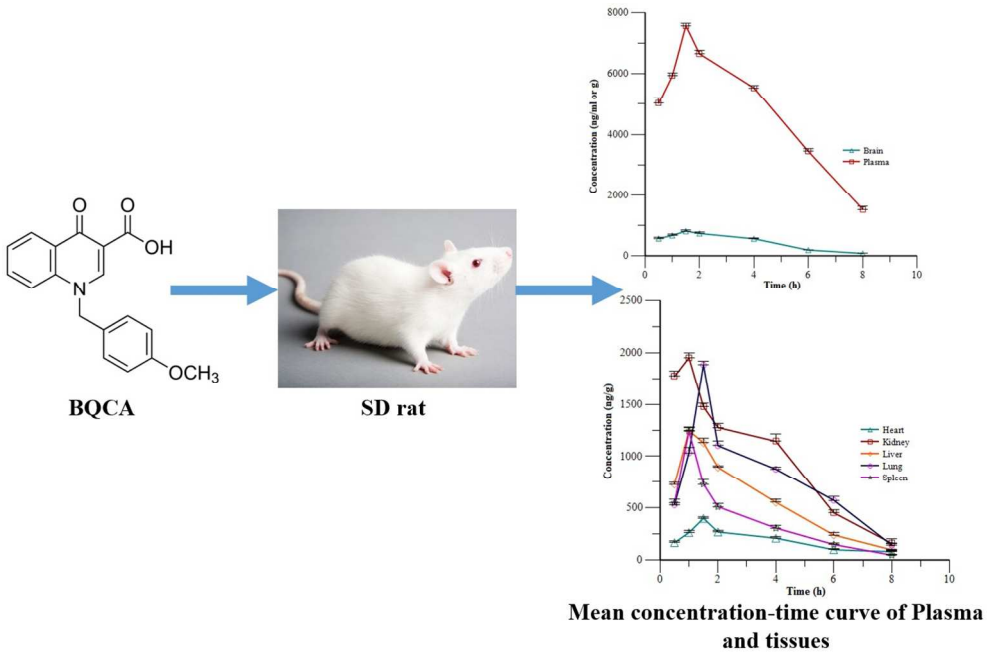

Mean concentration-time curve of Plasma and tissues

243x162mm (150 x 150 DPI)

Pharmacokinetics and Tissue distribution of a M1 Muscarinic Acetylcholine Receptor Positive Allosteric Potentiator, benzyl quinolone carboxylic acid

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ABSTRACT

A simple, sensitive and selective high performance liquid chromatography method has been developed and validated for the estimation of benzyl quinolone carboxylic acid (BQCA) in Sprague-Dawley (SD) rat plasma and tissue samples. Plasma and tissue samples were extracted by protein precipitation (PPT) technique using methanol as a precipitating agent and donepezil used as internal standard (IS). The chromatographic separation was performed on Hibar C_{18} column using acetonitrile and potassium dihydrogen orthophosphate buffer (20 mM, pH 6.5) at a flow rate of 0.8 mL/min. The lower limit of quantitation (LLOQ) of the developed method was found to be 2.0 ng/mL and 5.0 ng/g, respectively for plasma and tissue samples. BQCA remarkably absorbed into the systemic circulation with maximum concentration (~8000.0 ng/mL) within 1.5 h following *i.p.* administration. The order of AUC from the tissue distribution study was kidney> lung> liver> brain> spleen> heart. BQCA was meteorically taken up into the brain and bring off a maximal brain concentration at 1.5 h and maintained up to 3-4 h. The method was successfully applied for the analysis of BQCA in plasma and tissue samples followed by the *i.p.* administration of SD rats at 10mg/kg dose.

Keywords: M1 muscarinic acetylcholine receptor; Positive allosteric modulator; Benzyl quinolone carboxylic acid; Pharmacokinetics; Tissue distribution.

1. Introduction

Acetylcholinesterase inhibitors (AChEIs) such as donepezil, galantamine, rivastigmine and tacrine approved by the U.S. Food and Drug Administration (FDA) to treat Alzheimer's disease (AD), which act by limiting the degradation of synaptic acetylcholine (Ach) levels to activate cholinergic receptors [1,2]. However, AChEIs efficacy in enhancing cognition is failed so far, in part because of central and peripheral adverse effects that are due to activation of the other subtype of muscarinic Ach receptors (mAChRs) such as M2 to M5 and/or direct agonism action [3]. Recent research suggests that selective stimulation of M1 receptors, but not other subtypes, is a beneficial strategy to treat AD without producing adverse effects [4,5].

There are five receptor subtypes in the muscarinic family (M1-M5) that differ in terms of their localization, signalling activity and presumed function [6]. Among the five existing mAChR subtypes, M1 is predominant in the many memory related brain regions, including the cortex, hippocampus, and striatum [7]. Thus selective M1 receptor drug targeting could play important role in regulating higher cognitive function [8,9]. There is difficulty in developing highly selective M1 receptor agonists due to the high sequence homology among the orthosteric binding sites of mAChR subtypes [7]. The alternative novel approach is to achieve high subtype selectivity is by targeting allosteric binding sites that are distinct from the ACh binding sites. In this approach many G-protein-coupled receptors, including mAChRs [10,11], have allosteric binding sites bound by molecules that activate the receptor in the absence of ligand (allosteric agonist) or enhance the response to native ligand (positive allosteric modulator) [12].

Benzyl quinolone carboxylic acid (BQCA), [1-(4-methoxybenzyl)-4-oxo-1,4-dihydroquinoline- 3-carboxylic acid] is a novel highly selective potent positive allosteric modulator (PAM) of M1 receptor [13]. It reduces the concentration of ACh required to activate M1 as well as it has no effect on potentiation, agonism or antagonism activity on other subtypes of mAChRs and does not show unwanted peripheral cholinergic stimulation [(3,14]. BQCA enhances the memory formation and reverses the cognitive impairment in scopolamine-induced memory deficits, suggested that the M1 receptor PAM BQCA has therapeutic potential for the treatment of AD [3,7]. To date, there are limited published data on pharmacokinetic and tissue distribution studies of BQCA [6, 7]. The main purpose of present study is to report the detailed pharmacokinetics and tissue distribution of BQCA in Sprague-Dawley (SD) rats following intraperitoneal (i.p) administration at 10 mg/kg.

2. Experimental

2.1. Chemicals and reagents

BQCA (≥98%) as a gift sample was obtained from Vanderbilt Center for Neuroscience Drug Discovery (Nashville, USA) and Donepezil (IS, ≥98%) was gifted by Dr. Reddy's Laboratories Limited (Hyderabad, India). The corresponding structures are shown in Fig. 1. Acetonitrile (ACN) and methanol of HPLC grade were purchased from Merck (Mumbai, India), ortho-phosphoric acid and triethylamine of AR grade were procured from Qualigens Fine Chemicals (Mumbai, India), potassium dihydrogen orthophosphate was obtained from SD Fine Chemicals (Mumbai, India), polysorbate 80 was procured from sigma aldrich (St. Louis, USA) and HPLC grade water from Milli-Q RO system (Millipore India, Bangalore, India) were used.

2.2. Chromatographic conditions

Analysis of BQCA was performed on Shimadzu Prominence HPLC system (Shimadzu Corporation, Kyoto, Japan). HPLC was equipped with model series LC-20AT pump, Rheodyne 7752i injector with 20 µl loop and SPD- 20A UV/VIS detector. Separation was carried out on the Hibar C_{18} column (250 × 4.6 mm i.d., 5 µm) (Merck Limited., Mumbai). Spinchrom Chromatography station software performed the data acquisition. ACN and potassium dihydrogen orthophosphate buffer (20 mM, pH 6.5) at a ratio of 70:30 v/v was used as mobile phase at a flow rate of 0.8 mL/min with an injection volume of 20 µL and the detection wavelength was set at 215 nm. Prior to use, the mobile phase was filtered through a 0.22 µm hydrophilic membrane filter. All determinations were performed at room temperature (15-18ºC).

2.3. Preparation of calibration standards and quality control samples

Calibration standards were prepared by spiking the working standard solution into a pool of drug free rat plasma in order to obtain the following concentrations 2.0, 10.0, 50.0, 200.0, 1000.0, 5000.0, 20000.0 and 40000.0 ng/mL in plasma and 5.0, 50.0, 100.0, 500.0, 1000.0, 1500.0, 2000.0 and 5000.0 ng/g in tissues. These solutions were labelled and stored at -70 ± 2°C until analysis. Quality control samples (QCs) at a minimum of three concentrations for BQCA were prepared by spiking the working standard solutions into a pool of drug free SD rat plasma and tissues to produce a concentration of 6.0, 20000.0 and 32000.0 ng/mL and 5.0, 2500.0, and 4000.0 ng/g, respectively. These solutions were labelled and stored at -70 ± 2°C until the analysis.

2.4. Extraction of plasma and tissue samples

Protein precipitation (PPT) technique was generally the preferred choice of extraction technique because it is simple, economical, and less cumbersome. An aliquot of 500 µL of plasma, 500 µL of IS (100 µg/mL) and 500 µL of methanol was taken in to a 2 mL ependorff tube and vortexed for 30 s then centrifuged (Remi Instruments, Mumbai) at 10,000 rpm for 10 min. Clear supernatant was transferred into a vial and 20 µL was subjected to HPLC analysis. Tissue samples were weighed accurately and homogenized using a glass tissue homogenizer after addition of 1mL physiological saline. Tissue homogenates were processed similarly as plasma samples and analyzed by HPLC.

2.5. Method validation

The method validation of BQCA in SD rat plasma and tissue were performed according to the USFDA guidelines [16]. The assay was validated for specificity, extraction recovery, linearity, sensitivity, accuracy, precision, and stability.

2.5.1. Specificity

The specificity was established by the lack of interference peaks at the retention time of BQCA and the IS. Recovery was determined by comparing the mean peak area obtained from either the extracted plasma or tissue samples with the peak area obtained by the direct injection of the corresponding spiked standard solutions.

2.5.2. Extraction recovery

Recovery was determined by comparing the mean peak area obtained from either the extracted plasma or tissue samples with the peak area obtained by the direct injection of the corresponding spiked standard solutions. Different concentrations of BQCA (6.0, 20000.0, and 32000.0 ng/mL in plasma and 15.0, 2500.0, and 4000.0 ng/g in tissue samples) were measured.

2.5.3. Linearity and sensitivity

The linearity was tested over the concentration range of 2.0-40000.0 ng/mL in plasma and 10.0-5000.0 ng/g in tissues. The calibration curves were established by plotting the peak area ratio of BQCA to internal standard versus the BQCA concentration. The regression parameters of the slope, intercept and correlation coefficient were calculated by linear regression equation. The lowest limit of quantification (LLOQ) was set as the lowest amount of analyte in a sample that could be quantitatively determined with acceptable precision and accuracy (i.e., 20% coefficient of variation (CV) and ± 20% nominal concentration in these assays, respectively).

2.5.4. Accuracy and precision

The intra- and inter-day precision and accuracy in plasma and tissue samples were evaluated at three different QC levels in six replicates on the same day and on three different days, respectively. Acceptable deviation was set within 15% of the nominal concentration for accuracy and within 15% of the CV for precision.

2.5.5. Stability

Stability of BQCA in plasma and brain tissue were determined by the analysis of QCs (n=6) subjected to different storage conditions such as freeze thaw (3 cycles), short-term, long-term and stock solution stability. For freeze-thaw (3cycles) stability, the spiked plasma and tissue samples were frozen at -70°C for 24 h and thawed at room temperature. When completely thawed, the samples were refrozen for 12-24 h under the same conditions, at the end of each cycle samples were analysed and compared with the freshly prepared QCs (n=6) in plasma and tissue. For the short-term and stock solution stability study, plasma and tissue QCs were kept at 25ºC for 6 h and samples were processed, analysed and compared with the freshly prepared QCs. The long-term stability was evaluated following frozen of the plasma and tissue samples at -70 ± 2°C for 30 days. The samples were considered stable when the deviation from nominal values were within ±15%.

2.6. Pharmacokinetics and tissue distribution study

The pharmacokinetic and tissue distribution study were performed on Male SD rats weighing 180–220 g. The animal house was well ventilated and the animals were maintained on a 12:12 h light/dark cycle in large spacious cages throughout the experimental period. The animals were provided with food and water ad libitum and fasted for 12 h prior to the commencement of the experiment. The Institutional Animal Ethical Committee (IAEC) of JSS College of Pharmacy, Udhagamandalam, India, has been approved the study protocol (JSSCP/IAEC/PH.D/PH.BIOTECH/01/2012-13). Forty eight SD rats were randomly assigned into eight groups, each group contained six rats. BQCA was dissolved in normal saline (containing 1% polysorbate 80) and filtered through a 0.22 µm hydrophilic membrane filter and then administered to SD rats at a dose of 10 mg/kg through *i.p.* administration. Approximately 0.3 mL aliquot of blood samples were collected via heart puncture at time intervals of 0 min (pre dose), 0.5, 1.0, 1.5, 2.0, 4.0, 6.0, and 8.0 h of post dose. Interest of organs (heart, liver, spleen, lung, kidney and brain) were collected immediately after cervical dislocation at above described time points and weighed rapidly. Tissues were kept into normal saline solution to remove blood or content and blotted dry with tissue paper. Blood and tissue samples were centrifuged at 10,000 rpm for 10 min and supernatants were collected and stored at -70 ± 2ºC until analysis.

The pharmacokinetic parameters were calculated by non-compartmental analysis of individual concentration-time data using Phoenix WinNonlin® v 6.3 (Pharsight Corporation, Mountain view, CA, USA). The pharmacokinetic parameters such as maximum plasma concentration (C_{max}) and the time to reach C_{max} (T_{max}) were obtained directly from the plasma concentration-time curve. Elimination rate constant (K_{el}) was obtained from the least-square fitted terminal log-linear portion of the plasma concentration-time profile, elimination half-life ($T_{1/2}$) was calculated as 0.693/ K_{el}, area under the plasma concentration time curve from 0 to 8 h ($AUC_{0\text{-}8h}$) was calculated by the linear trapezoidal rule, and area under the curve from 0 h extrapolated to infinity ($AUC0_{0\text{-}\infty}$) was calculated as $AUC_{0\text{-}8h} + C_t/K_{el}$ where C_t represent the observed plasma concentration at the last measurable sampling time. The apparent clearance (CL/F) was calculated as the drug dose divided $AUC_{0\text{-}8h}$ and apparent volume of distribution (Vz/F) was calculated as CL/F divided by K_{el}. All values are expressed as means ± Standard Deviation except the Tmax, which is expressed as the median.

3. Results and discussion

3.1. HPLC analysis

As the molecular weight of the BQCA is less than 2000 Da and extreme polar nature, reverse phase mode was used for the analysis with C_{18} column [15]. A better resolution factor was observed at the ratio of 70% ACN and 30% potassium dihydrogen orthophosphate (20 mM, pH 6.5). Initially, at flow rate of 1mL/min, the mobile phase of above ratio reported poor resolution, as BQCA was merged with IS. To increase the resolution of IS, flow rate of mobile phase was reduced to 0.8 mL/min that results the run time of 10 min with retention time of 4.9 and 7.6 min, respectively for BQCA and IS. No attempt was made for further reduction of the mobile phase flow rate as it may increase the analysis runtime. BQCA was highly sensitive to the wavelength of 215nm. During the process of validation, solid phase extraction, liquid-liquid extraction and protein precipitation technique were applied to determine the limit of detection for BQCA. The solid phase extraction (SPE) technique reported the limit of detection of 0.002 and the protein precipitation (PPT) technique also reported the same limits when methanol was used as a protein precipitation agent. The liquid-liquid extraction (LLE) technique accounts much higher limit of detection for BQCA. Thus the further validation of method was carried by PPT technique since the SPE is time consuming with multi purification steps.

3.2. Method validation

3.2.1. Specificity

The specificity of a method can be defined as the extent to which the analyte can be estimated without the intervention of other components. BQCA and IS were very well resolved under the proposed chromatographic conditions. None of the drug free plasma and tissue samples studied in this assay yielded endogenous interference at the retention time observed for drug. Fig. 2 represents the standard HPLC chromatogram of blank plasma, tissue and spiked plasma, tissue.

3.2.2. Extraction recovery

The percentage mean recovery of BQCA in plasma was ranged from 94.43-95.69% and in brain ranged from 91.57-96.52%. Recovery of IS at 100 µg/mL was found to be 94.15 and 92.76% in plasma and tissue, respectively. Table 1 summarizes the extraction recoveries for the plasma and various tissue samples.

3.2.3. Linearity and sensitivity

The linearity of each calibration curve was determined by plotting response factor and concentration of the standard solution. A linear calibration curves of BQCA in plasma and tissue were observed at 2.0-40000.0 ng/mL and 5.0-5000.0 ng/g, respectively. The results of linear regression analysis were listed in Table 2 and showed that the correlation coefficients of the calibration curves for all sample types were greater than 0.99. LLOQ in plasma and tissues was tested at different levels ranging from 2.0 to 100.0 ng/mL or g and it was found to be 2.0 ng/mL and 5.0 ng/g, respectively, with an accuracy of 92.43% with 5.8% of precision in plasma and 90.78% of accuracy with 6.7% of precision in tissues. The results indicating for LLOQ of BQCA was with in the acceptable precision and accuracy range.

3.2.4. Accuracy and precision

The accuracy and precision of intra-and inter-day studies at three different QCs of BQCA in plasma and tissues were found to be within the acceptable limits. The results indicated that the assay method was accurate and precise for replicate analysis of BQCA in plasma and tissues and results were summarized in Table 1.

3.2.5. Stability

The stability of BQCA in plasma and brain were evaluated by measuring concentration change in QCs (n = 3) under different storage conditions, the results indicated that BQCA was stable at all the tested stability conditions. Table 3 depicts the percentage changes in the mean concentration of BQCA under all the conditions tested.

3.3. Pharmacokinetic study

A validated method was applied for quantitative estimation of BQCA in plasma samples obtained followed by the *i.p.* administration at a dose of 10 mg/kg. The mean plasma concentration-time profile for BQCA was shown in Fig. 3a, and pharmacokinetic parameters were listed in Table 4. BQCA remarkably absorbed into the systemic circulation with maximum concentration (~8000.0 ng/mL) at 1.5 h following *i.p.* administration. Mean peak plasma concentration of BQCA was 7574.21 ± 82.01 ng/mL and its plasma concentration after four half-lives was ~473.39 ng/mL. Since the method LLOQ was 2.0 ng/mL for BQCA, its sensitivity was adequate for the bioavailability studies. Pharmacokinetic parameters in plasma such as C_{max}, $AUC_{(0\text{-}8h)}$, $AUC_{(0\text{-}\infty)}$, CL/F, K_{el}, Vz/F, $T_{max,}$ $T_{1/2}$ and MRT were found to be 7574.21 ± 82.01 ng/mL, 36610.64 ± 293.15 h ng/mL, 45876.05 ± 787.02 h ng/mL, 218.03 ± 3.7 mL/h/kg, 0.16 ± 0.01 1/h, 1324.19 ± 25.36 mL/kg, 1.5 h, 4.21 ± 0.07 h and 3.42 ± 0.02 h, respectively.

3.4. Tissue distribution study

Concentrations of BQCA were determined in various tissues of SD rat such as brain, heart, lung, liver, spleen, and kidney. Fig. 3b shows the concentration-time curve of BQCA in various tissues following *i.p.* administration at 10 mg/kg in SD rats. BQCA meteorically taken up into the brain and bring off a maximal concentration at 1.5 h and maintained up to 3-4 h. Pharmacokinetic parameters in brain such as C_{max}, $AUC_{(0\text{-}8h)}$, $AUC_{(0\text{-}\infty)}$, CL/F, K_{el}, Vz/F, $T_{max,}$ $T_{1/2}$ and MRT were found to be 808.57 ± 32.11 ng/g, 3412.93 ± 114.74 h ng/mL, 3660.01 ± 140.76 h ng/mL, 2735.56 ± 103.54 g/h/kg, 0.31 ± 0.02 1/h, 8883.73 ± 527.12 g/kg, 1.5 h, 2.25±0.11 h and 2.99 ± 0.04 h, respectively. Pharmacokinetic tissue distribution of BQCA was analysed by non-compartment model and the parameters were presented in Table 5. The concentration of BQCA was low in all collected tissues at 8 h. The order of AUC was kidney> lung> liver> brain> spleen> heart. The order of maximum BQCA concentration was kidney> lung> liver> spleen> brain> heart. The maximum concentration (1951.8 ng/g) was found in the kidney suggested that renal excretion may be the main BQCA elimination pathway due to the increased drug exposure.

4. Conclusion

A sensitive, rapid HPLC method was developed and validated for the estimation of BQCA in SD rat plasma. A simple PPT technique was employed to analyse the plasma and tissue samples. The present method showed high sample throughput and greater sensitivity (2.0 ng/mL in plasma and 5.0 ng/g in tissues). This work provides the data for plasma

pharmacokinetics and tissue distribution of BQCA in SD rats. The achieved pharmacokinetics and tissue distribution results may useful for further study of the bioactive mechanism of BQCA. The method was successfully applied for analysis of BQCA in plasma and tissue samples followed by *i.p.* administration at 10 mg/kg.

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Table Captions

Table 1 Recovery, accuracy and precision for BQCA in plasma and tissues (n=6).

Table 2 Equation of linear regression analysis.

Table 3 Summary of stability studies of BQCA in rat plasma under various storage conditions (n = 6).

Table 4 Mean pharmacokinetic parameters of BQCA in plasma and brain following i.p. administration at 10 mg/kg.

Table 5 The main pharmacokinetic parameters of BQCA in visceral organs following i.p. administration at 10 mg/kg.

Table 1 Recovery, accuracy and precision for BQCA in plasma and tissues (n=6).

Biological Sample	QCs (ng/mL or g)	Concentration found (ng/mL or g)	Recovery (%)	Intra-day		Inter-day	
				Accuracy (% Nominal)	Precision (% CV)	Accuracy (% Nominal)	Precision (% CV)
Plasma	6.0	5.7 ± 0.24	94.43	95.81	4.2	91.59	5.6
	20000.0	19138 ± 880.35	95.69	94.54	4.6	92.21	6.2
	32000.0	30406.4 ± 1489.91	95.02	95.07	4.9	90.35	5.9
Brain	15.0	13.73 ± 0.92	91.57	94.43	6.7	90.56	6.8
	2500.0	2391.75 ± 138.72	95.67	95.84	5.8	92.38	6.5
	4000.0	3860.8 ± 216.21	96.52	95.32	5.6	92.11	6.4
Liver	15.0	13.94 ± 0.86	92.91	95.14	6.2	91.09	6.9
	2500.0	2371.25 ± 144.65	94.85	94.86	6.1	90.27	5.4
	4000.0	3786.4 ± 215.82	94.66	96.47	5.7	92.55	5.8
Lung	15.0	13.97 ± 0.95	93.14	93.24	6.8	90.02	5.9
	2500.0	2344.5 ± 107.85	93.78	95.62	4.6	92.16	5.7
	4000.0	3832.8 ± 206.97	95.82	94.08	5.4	91.23	6.2
Kidney	15.0	14.05 ± 0.83	92.69	95.68	5.9	91.44	6.6
	2500.0	2335.25 ± 121.43	93.41	95.71	5.2	91.59	6.3
	4000.0	3669.2 ± 168.78	91.73	96.22	4.6	90.83	5.2
Spleen	15.0	13.55 ± 0.91	90.32	93.91	6.7	90.17	6.1
	2500.0	2287 ± 118.92	91.48	94.97	5.2	90.25	5.8
	4000.0	3652.8 ± 211.86	91.32	94.83	5.8	91.08	5.8
Heart	15.0	13.79 ± 0.86	91.93	95.36	6.2	90.27	6.4
	2500.0	2316 ± 111.17	92.64	95.39	4.8	91.55	6.2
	4000.0	3715.2 ± 170.9	92.88	94.25	4.6	90.63	5.6

Table 2 Equation of linear regression analysis.

Biological Sample	Concentration range (ng/mL or g)	Equation	R^2
Plasma	2.0-40000.0	y = 0.00002x + 0.01075	0.999
Brain	5.0-5000.0	y = 0.1174x + 0.0128	0.998
Liver	5.0-5000.0	y = 0.1338x + 0.0153	0.998
Lung	5.0-5000.0	y = 0.1289x + 0.0123	0.999
Kidney	5.0-5000.0	y = 0.0899x + 0.0071	0.999
Spleen	5.0-5000.0	y = 0.0962x + 0.0048	0.999
Heart	5.0-5000.0	y = 0.0716x + 0.0024	0.998

Table 3 Summary of stability studies of BQCA in rat plasma under various storage conditions (n = 6).

Stability test	Biological sample							
	Plasma				Brain			
	QCs (ng/mL)	Mean ± SD (ng/mL)	Accuracy (%Nominal)	Precision (%CV)	QCs (ng/g)	Mean ± SD (ng/g)	Accuracy (%Nominal)	Precision (%CV)
Freeze-thaw (3 cycles at -70 ± 2ºC)	6.0	5.38 ± 0.33	89.68	6.2	15.0	13.52 ± 0.85	90.12	6.3
	20000.0	18092 ± 1031.24	90.46	5.7	2500.0	2281 ± 139.14	91.24	6.1
	32000	29372.8 ± 1703.62	91.79	5.8	4000.0	3656.8 ± 215.75	91.42	5.9
Short-term (at 25ºC for 6h)	6.0	5.55 ± 0.34	92.45	6.1	15.0	13.87 ± 0.81	92.47	5.8
	20000.0	18722 ± 1160.76	93.61	6.2	2500.0	2289.5 ± 132.79	91.58	5.8
	32000.0	29852.8 ± 1761.32	93.29	5.9	4000.0	3735.6 ± 209.19	93.39	5.6
Long-term (at -70 ± 2ºC for 1 month)	6.0	5.32 ± 0.36	88.68	6.8	15.0	13.17 ± 0.88	87.79	6.7
	20000.0	17412 ± 1131.78	87.06	6.5	2500.0	2201 ± 136.46	88.04	6.2
	32000.0	28588.8 ± 1858.27	89.34	6.5	4000.0	3524.8 ± 229.11	88.12	6.5
Stock solution (at 25ºC for 6h)	6.0	5.87 ± 0.27	97.89	4.6	15.0	14.24 ± 0.59	94.95	4.2
	20000.0	19304 ± 733.55	96.52	3.8	2500.0	2417.75 ± 108.79	96.71	4.5
	32000.0	30966.4 ± 1269.61	96.77	4.1	4000.0	3835.6 ± 180.27	95.89	4.7

Table 4 Mean pharmacokinetic parameters of BQCA in plasma and brain following i.p. administration at 10 mg/kg.

Parameters	Plasma	Brain
C_{max} (ng/mL or g)	7574.21 ± 82.01	808.57±32.11
$AUC_{(0\text{-}8h)}$ (h ng/mL or g)	36610.64±293.15	3412.93± 114.74
$AUC_{(0\text{-}\infty)}$ (h ng/mL or g)	45876.05±787.02	3660.01± 140.76
CL/F (mL or g/h/kg)	218.03±3.7	2735.56±103.54
K_{el} (1/h)	0.16±0.01	0.31±0.02
Vz/F (mL or g/kg)	1324.19±25.36	8883.73±527.12
T_{max} (h)	1.5	1.5
$T_{1/2}$ (h)	4.21 ± 0.07	2.25±0.11
MRT (h)	3.42 ± 0.02	2.99± 0.04

Table 5 The main pharmacokinetic parameters of BQCA in visceral organs following i.p. administration at 10 mg/kg.

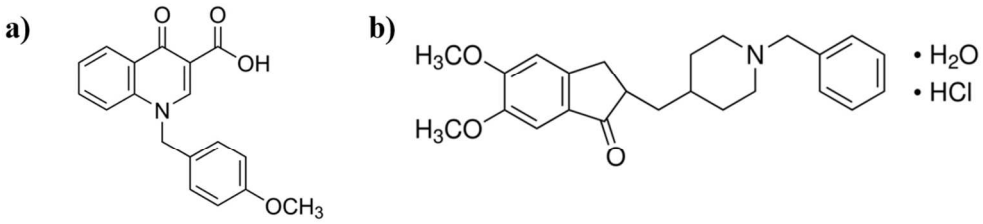
Parameters	Heart	Kidney	Liver	Lung	Spleen
C_{max} (ng/g)	398.56 ± 15.75	1951.8 ± 49.44	1244.25 ± 37.08	1883.25 ± 36.98	1239.50±37.36
$AUC_{(0\text{-}8h)}$ (h ng/g)	1419.36 ± 26.80	7373.95 ± 236.50	4251.01 ± 102.82	5989.59 ± 59.04	2774.81± 79.13
$AUC_{(0\text{-}\infty)}$ (h ng/g)	1701.51 ± 59.50	8038.46 ± 440.21	4543.22 ± 117.88	6621.28 ± 86.87	2910.92 ± 98.50
CL/F (g/h/kg)	5883.289 ± 211.19	1247.11 ± 67.84	2202.33 ± 57.45	1510.50 ± 19.80	3438.69 ± 118.61
K_{el} (1/h)	0.28 ± 0.02	0.24 ± 0.01	0.33 ± 0.02	0.23 ± 0.02	0.33± 0.03
Vz/F (g/kg)	21025.23 ± 1176.04	5161.03 ± 245.53	6656.71 ± 382.91	6703.33 ± 515.54	10366.41± 1233.78
T_{max} (h)	1.5	1	1	1.5	1
$T_{1/2}$ (h)	2.48 ± 0.16	2.87 ± 0.17	2.09 ± 0.17	3.08 ± 0.26	2.09±0.19
MRT (h)	3.28 ± 0.06	2.9 ± 0.07	2.83 ± 0.06	3.28 ± 0.05	2.59±0.04

Figure Captions

Fig. 1. Molecular structures of (a) BQCA and (b) donepezil (IS).

Fig. 2. Representative chromatograms of a) blank plasma, b) plasma spiked with BQCA and IS, c) plasma sample after *i.p.* administration of BQCA, d) blank tissue of brain; e) brain tissue spiked with BQCA and IS, f) brain tissue sample after *i.p.* administration of BQCA.

Fig. 3. Concentration-time curve of a) plasma and b) tissues in rats followed by the *i.p.* administration of BQCA at 10 mg/kg (Mean ± SD).



Molecular structures of (a) BQCA and (b) donepezil (IS).
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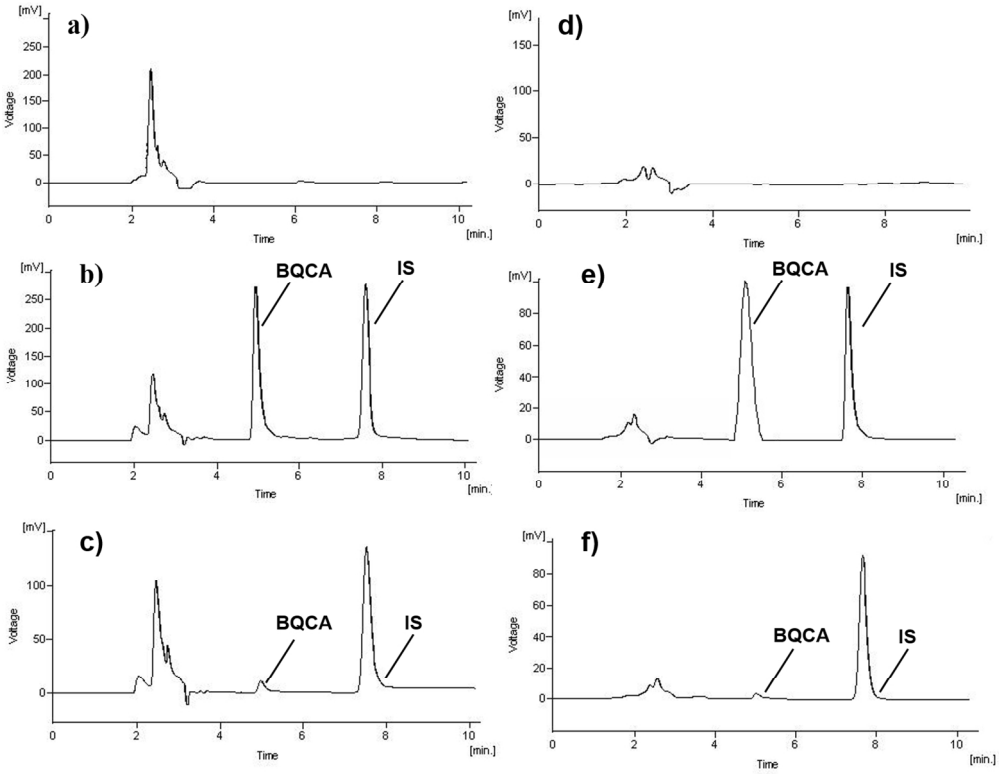
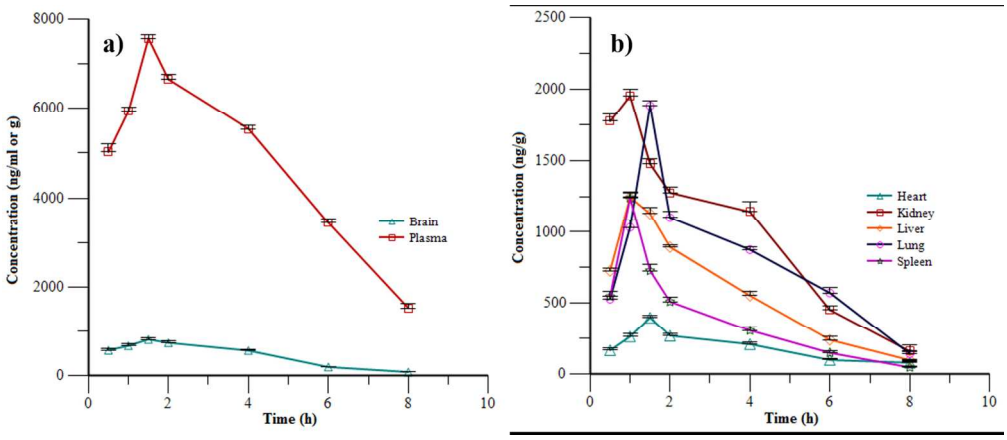


Representative chromatograms of a) blank plasma, b) plasma spiked with BQCA and IS, c) plasma sample after i.p. administration of BQCA, d) blank tissue of brain; e) brain tissue spiked with BQCA and IS, f) brain tissue sample after i.p. administration of BQCA.
401x317mm (96 x 96 DPI)



Concentration-time curve of a) plasma and b) tissues in rats followed by the i.p. administration of BQCA at 10 mg/kg (Mean ± SD).
273x117mm (150 x 150 DPI)